Figure 8: Histologic appearance of lung tissue from control and thapsigargin-treated wild type mice. Sections of lung tissue from untreated (A) and thapsigargin-treated (B and C) mice were stained with hematoxylin and eosin. The scale bar in panel C = 280μ.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

A. Definitions

Antisense. The term "antisense", as used herein, refers to nucleotide sequences that are complementary to a specific DNA or RNA sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules may be produced by any method, including synthesis by ligating the gene(s) of interest in a reverse orientation to a viral promoter which permits the synthesis of a complementary strand. Once introduced into a cell, this transcribed strand combines with natural sequences produced by the cell to form duplexes. These duplexes then block either the further transcription or translation. In this manner, mutant phenotypes may be generated. The designation "negative" is sometimes used in reference to the antisense strand, and "positive" is sometimes used in reference to the sense strand.

Clinical Condition. Any symptom or disorder related to any disease.

Combinatorial Chemistry. "Combinatorial chemistry," as used herein, refers to the numerous technologies used to create hundreds or thousands of chemical compounds, wherein each of the chemical compounds differ for one or more features, such as their shape, charge, and/or hydrophobic characteristics.

Disease. A pathological condition of a cell, body part, an organ, a tissue, or a system resulting from various causes, wherein such causes include, but are not limited to, infections, genetic defects or environmental stresses.

Mis-assembled. As used herein, "mis-assembled" refers to hetero- or homo-oligomeric proteins that have not or can not attain their appropriate or functionally mature quaternary structure and/or to hetero- or homo-oligomeric proteins that have a three-

dimensional structure different to wild type that causes retention in the ER or in an ER-Golgi compartment.

Mis-folded. As used herein, "mis-folded" refers to proteins that have not or can not attain their appropriate or functionally mature tertiary structure and/or to hetero- or homo-oligomeric proteins that have a three-dimensional structure different to wild type that causes retention in the ER or in an ER-Golgi compartment.

Nebulized. As used herein, "nebulized" refers to converting a liquid to a fine spray. A medicated spray is one form of the nebulization of a liquid.

Nucleic Acid Sequence. "Nucleic acid sequence," as used herein, refers to an oligonucleotide, nucleotide, or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represents the sense or antisense strand. Similarly, "amino acid sequence" as used herein refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragments or portions thereof, and to naturally occurring or synthetic molecules.

Treating. As used herein, "treating" includes reversing, alleviating, inhibiting the progress of, preventing, or reducing the likelihood of the disease, disorder, or condition to which such term applies, or one or more symptoms or manifestations of such disease, disorder or condition.

UGGT. As used herein, "UGGT" refers to UDP-Glc:glycoprotein glycosyl transferase, also known as UDP glycoprotein glycosyl transferase and as UDP-glucose:glycoprotein glucosyl transferase. UGGT is an ER enzyme that attaches glucose to malformed/improperly folded glycoproteins, but not to wild type glycoproteins.

B. <u>Elevation of cyclic AMP Levels.</u> As discussed above, CFTR is a cAMP-dependent chloride channel. Cyclic AMP is composed of adenosine monophosphate with the phosphate group bonded internally to form a cyclic molecule. Cyclic AMP (cAMP) is generated from adenosine triphosphate (ATP) by the enzyme adenylcyclase and is active in the regulation of gene expression of both prokaryotes and eukaryotes.

Administration of compositions that increase or supplement the cAMP levels of epithelial cells has been used in an attempt to activate Cl^- conductance to near wild type levels (U.S. Patent No. 5,434,086). A preferred compound for increasing cAMP levels is a phosphodiesterase inhibitor, such as methylxanthine phosphodiesterase inhibitor. Phosphodiesterase inhibitors increase cAMP levels by inhibiting cAMP breakdown. Other examples of phosphodiesterase inhibitors include nonspecific inhibitors such as

alkylxanthines and cAMP-specific inhibitors such as Rolipram (Shearing AG). Preferred alkylxanthines include the methylxanthines, such as 3-isobutyl-1-methylxanthine (IBMX) and 1,3-dimethylxanthine (theophylline) and other xanthines such as papaverine, pentoxifilline and caffeine. For a review of phosphodiesterase inhibitors, *see* Nicholson *et al.*, Trends Pharmacol. Sciences 12:19 (1991) and Beavo *et al.*, Trends Pharmacol. Sciences 11:150 (1990).

Treating ΔF508-C127 cells and human ΔF508 airway epithelial cells with a carboxylic acid or a carboxylate, such as butyrate (*e.g.*, sodium butyrate), resulted in the generation of cAMP-dependent chloride channel activity (U.S. Patent No. 5,674,898).

Supplemental cAMP and analogs thereof or beta andrenergic receptor agonists, such as isoproterenol and albuterol, can also be used to increase cAMP levels.

Guanosine monophosphate (GMP) becomes a cyclic molecule by a phosphodiester bond between the 3' and 5' atoms. Cyclic GMP (cGMP) acts at the cellular level as a regulator of various metabolic processes, possibly as an antagonist to cAMP.

Combination therapy that includes administration of an inhibitor specific for a cGMP-inhibited type III cAMP phosphodiesterase, an adenylate cyclase activator, and a cAMP or a cAMP analog has also been proposed for treating CF (U.S. Patent No. 5,602,110). Inhibitors which are specific for a cGMP-inhibited type III cAMP phosphodiesterase include amrinone, milrinone, anagrelide, cilostamide and fenoxamine. Adenylate cyclase activators include forskolin, cholera toxin and beta-adrenergic receptor agonists.

C. Calcium-ATPase Inhibitors. Correct distribution of Ca^{+2} ions within the cellular compartments is required for their well-established function as molecular signals in eukaryotic cells (Cheek, T. R., Curr. Opin. Cell. Biol. 3:199-205 (1991); Pietrobon *et al.*, Eur. J. Biochem. 193:599-622 (1990)). ATP-dependent Ca^{+2} uptake from the cytosol to ER lumen is a prerequisite for rapid cytostolic signaling through receptor-mediated Ca^{+2} release (Berridge, M.J., Nature 361:315-325 (1993)).

The ATP-requiring Ca^{+2} transport to the ER lumen is accomplished by a family of ER Ca^{+2} ATPases termed SERCA ATPases. Ca^{+2}-ATPase inhibitors may be therapeutically useful in treating CF by improving Cl^- secretion in epithelial cells. Proposed Ca^{+2}-ATPase inhibitors for use in the present invention, include, but are not limited to, thapsigargin, cyclopiazonic acid (CPA) and 2,5-di-(tert-butyl)-1,4-hydroquinone (DBHQ) (A.C. Chao *et al.*, J. Clin. Invest. 96(4):1794-1801 (1995) and U.S. Patent No. 5,384,128). Thapsigargin is described in more detail below. CPA is an indole derivative